DBS DNA Extraction and Quantitation

NBS Molecular Training Class
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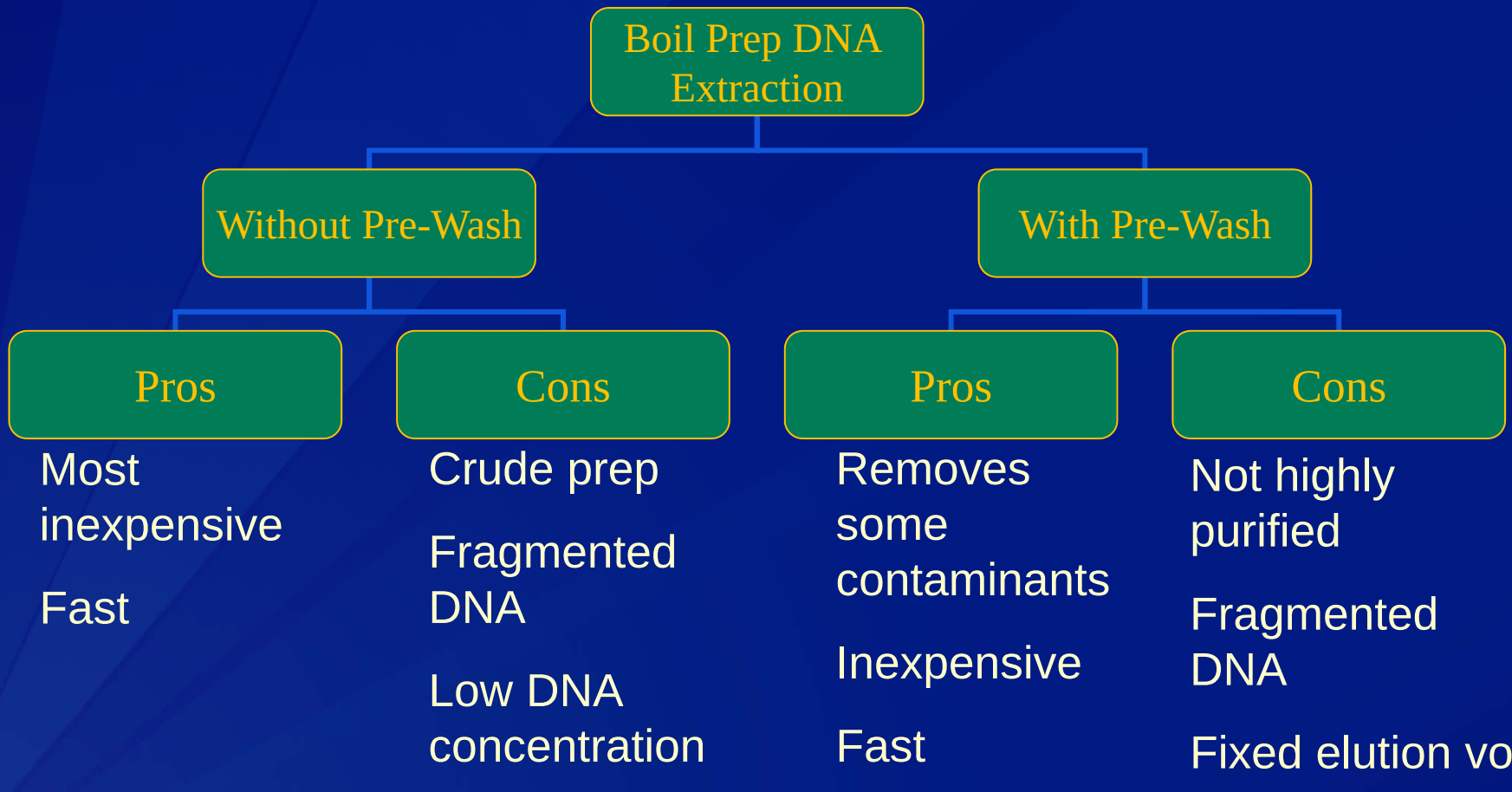
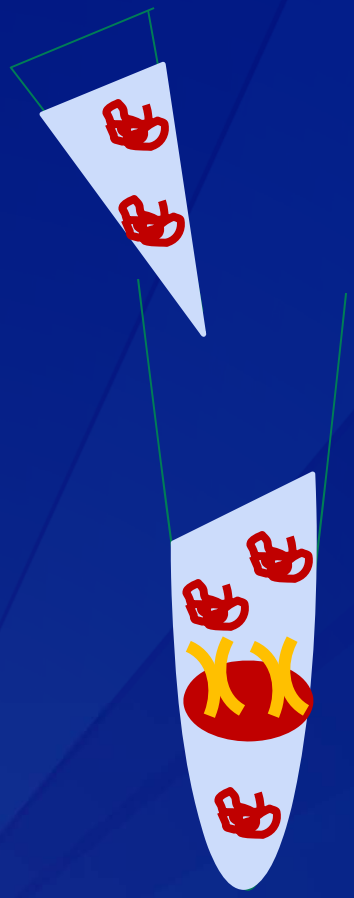
Selecting a DBS DNA Extraction Method

- Boiling Procedure
  - With or without an initial wash
- Highly Purified Extraction
  - Column Based Purification
  - Magnetic Bead Based Purification

Boiling DNA Extraction Methods for DBS



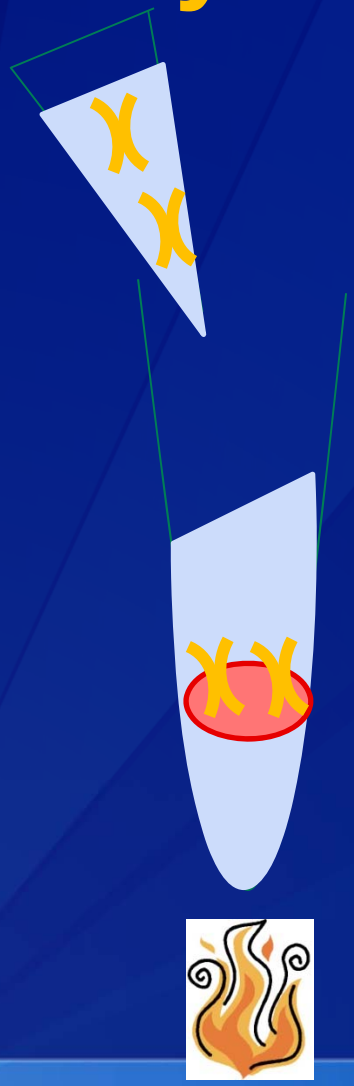
Commonly Used Boiling with a Pre-Wash: Lysis DNA Extraction



Step 1:
Add cell lysis solution to break open cell.

Remove supernate to wash away contaminants including heme & other proteins

Boiling with a Pre-Wash: Lysis DNA Extraction cont.

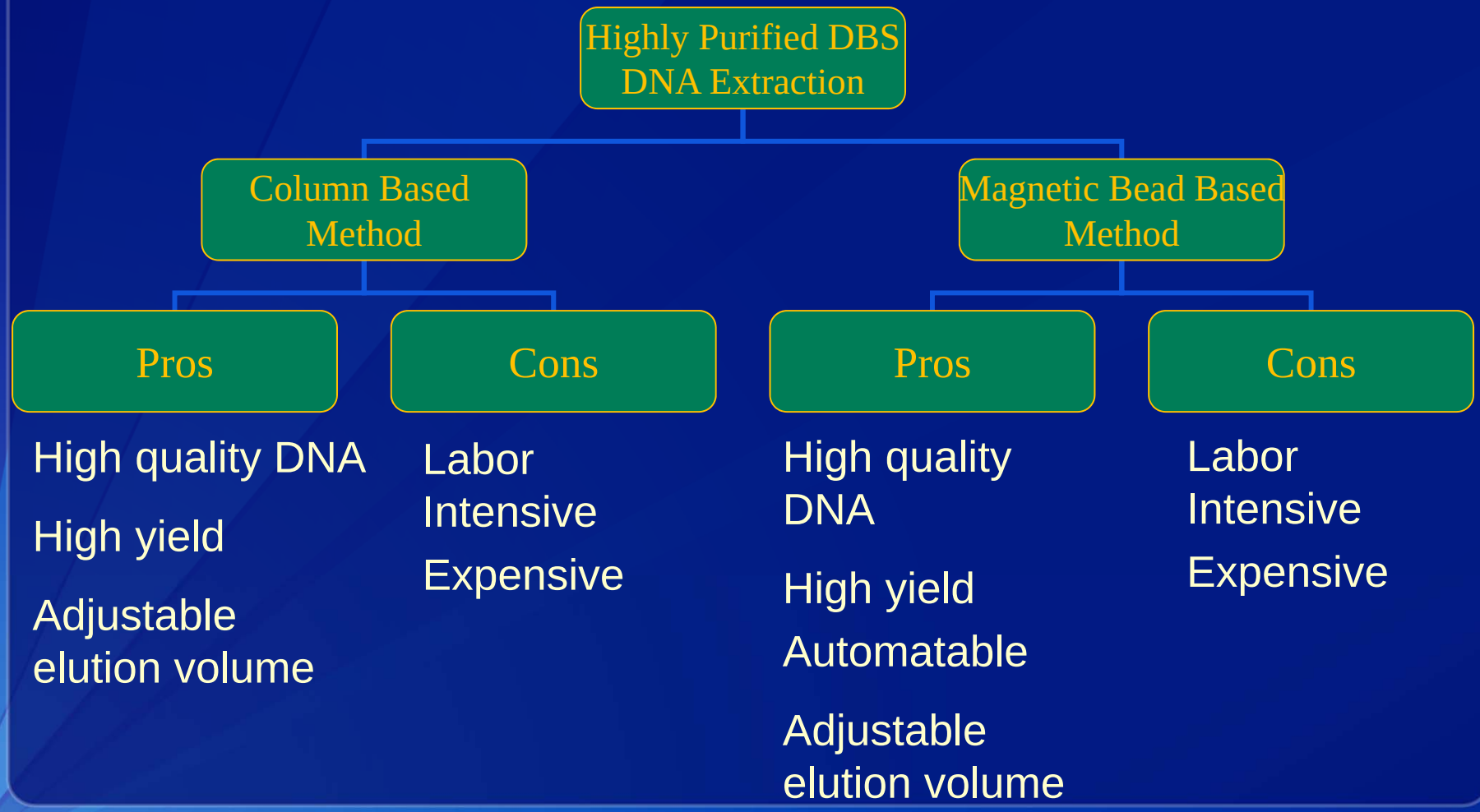


Step 1:
Add cell lysis solution to break open cell.

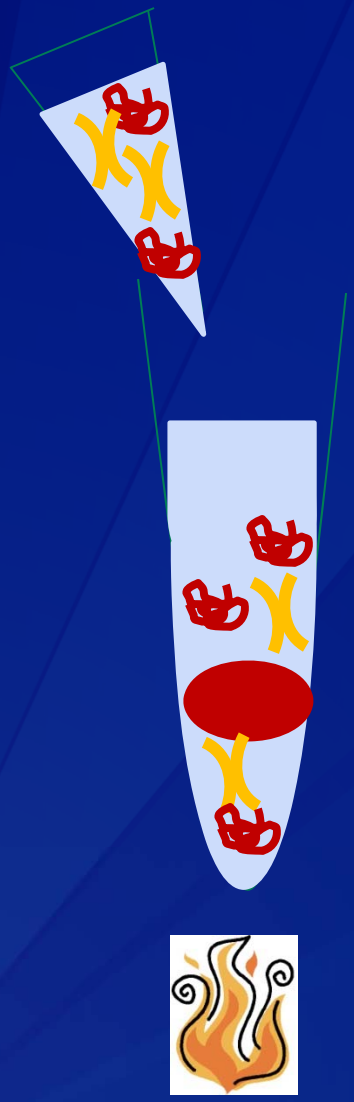
Remove supernate to wash away contaminants including heme & other proteins

Step 2:
Add DNA elution solution AND heat to remove DNA from the DBS.

Highly Purified DNA Extraction Methods for DBS



Column-based DNA Extraction



Step 1:
Add cell lysis solution to break open cell and heat to remove everything from the DBS.

Add binding buffer so the DNA will stick to the column matrix.

Remove entire mixture to be put in column.

Column-based DNA Extraction cont.

Step 1:
Add cell lysis solution to break open cell and heat to remove everything from the DBS.

Add binding buffer so the DNA will stick to the column matrix.

Remove entire mixture to be put in column.

Step 2:
Add lysed cell mixture to filter matrix.

Column-based DNA Extraction cont.

Step 1:
Add cell lysis solution to break open cell and heat to remove everything from the DBS.

Add binding buffer so the DNA will stick to the column matrix.

Remove entire mixture to be put in column.

Step 2:
Add lysed cell mixture to filter matrix. Centrifuge column to push proteins through the matrix keeping DNA in column.

Column-based DNA Extraction cont.

Step 1:
Add cell lysis solution to break open cell and heat to remove everything from the DBS.

Add binding buffer so the DNA will stick to the column matrix.

Remove entire mixture to be put in column.

Step 2:
Add lysed cell mixture to filter matrix.

Centrifuge column to push proteins through the matrix keeping DNA in column.

Step 3:
Add DNA elution reagent to detach from filter and centrifuge into tube.

DBS DNA Quantitation: When and How?

- Typically unnecessary for routine PCR based assays
- Important for validating new assay limits and sensitivity
  - Too little DNA may lead to allele drop-out (not always obvious)
  - Some assays require a minimum DNA quantity

DBS DNA Quantitation: When and How cont.

- ***Absorbance***
  - Measure not specific to DNA
  - DBS DNA contains contaminants resulting in inaccurate measures
  - Not recommended for DBS DNA
- ***Pico-green***
  - Measure specific to double stranded DNA
  - Recommended for DBS DNA
- ***Quantitative PCR***
  - Measure specific to amplifiable DNA
  - PCR inhibitors may underestimate DNA concentration
  - Different genomic targets may give different concentrations
  - Recommended for DBS DNA

DNA Quantitation: Absorbance

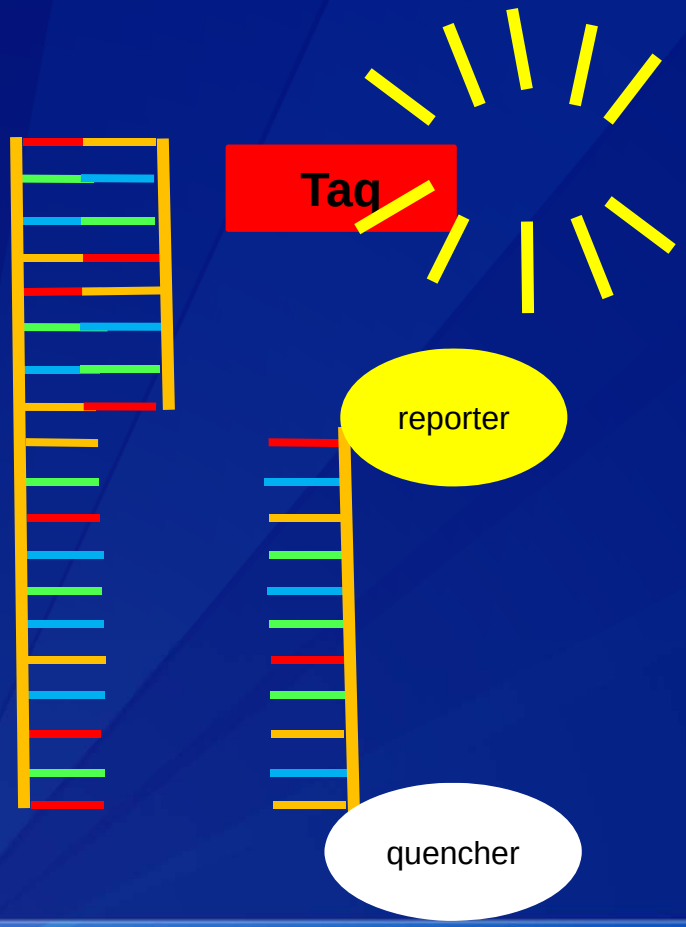
- DNA absorbs UV light at 260nm
- Spectrophotometer reads the amount of light that passes through the sample to determine the amount of DNA present
- Disadvantage: cannot distinguish between dsDNA, ssDNA, RNA or aromatic organic compounds
- Proteins absorb UV light near 280nm
- A sample with little protein contamination will have A260/280 ratio of 1.8.

DNA Quantitation: Picogreen

- Fluorescent dye binds to dsDNA
- Absorbs light at 480nm (blue) and emits light at 520nm (green)
- Using a known standard curve, the amount of light emitted can be used to calculate DNA quantity
- Unincorporated dye does not absorb light at 480nm
- Contaminants typically do not impact this measure

DNA Quantitation: quantitative PCR

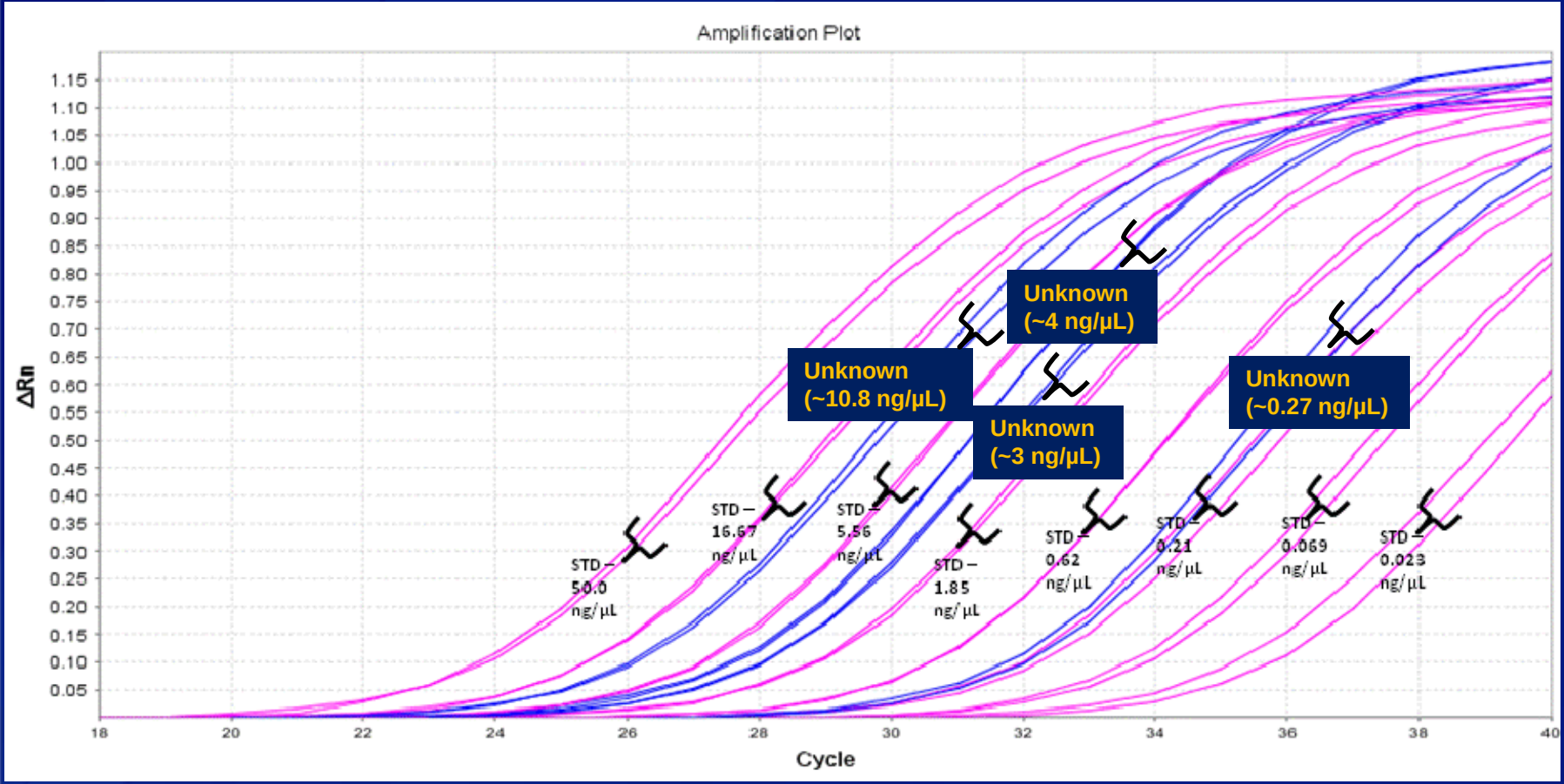
DNA florescence is measured during each cycle of amplification which is used to calculate quantity.



The fluorescent labeled probe anneals to the genomic DNA. The label is not visible prior to amplification.

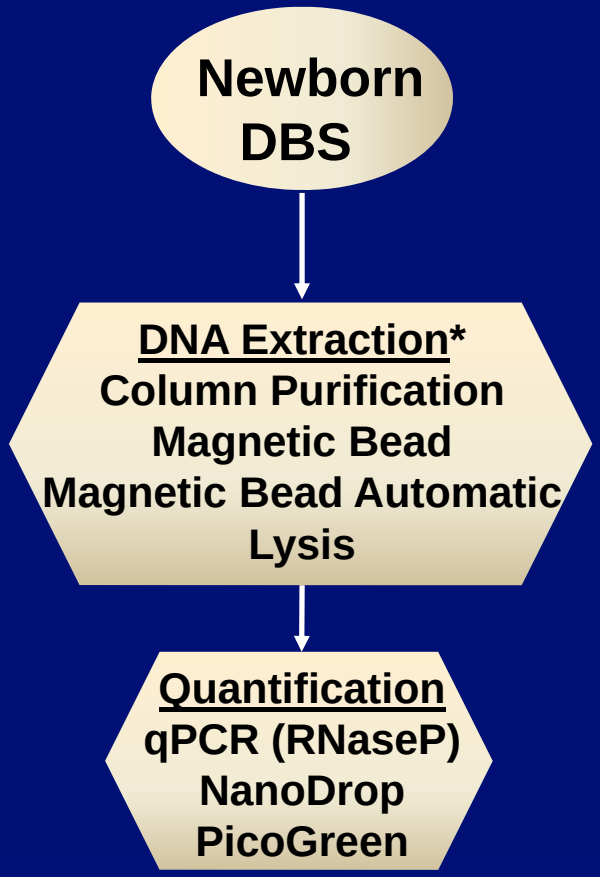
Taq polymerase begins to synthesize the new DNA strand. Once the enzyme encounters the probe, the exonuclease activity of the Taq will degrade the probe allowing visualization of the label.

qPCR: RNaseP Amplification Plot



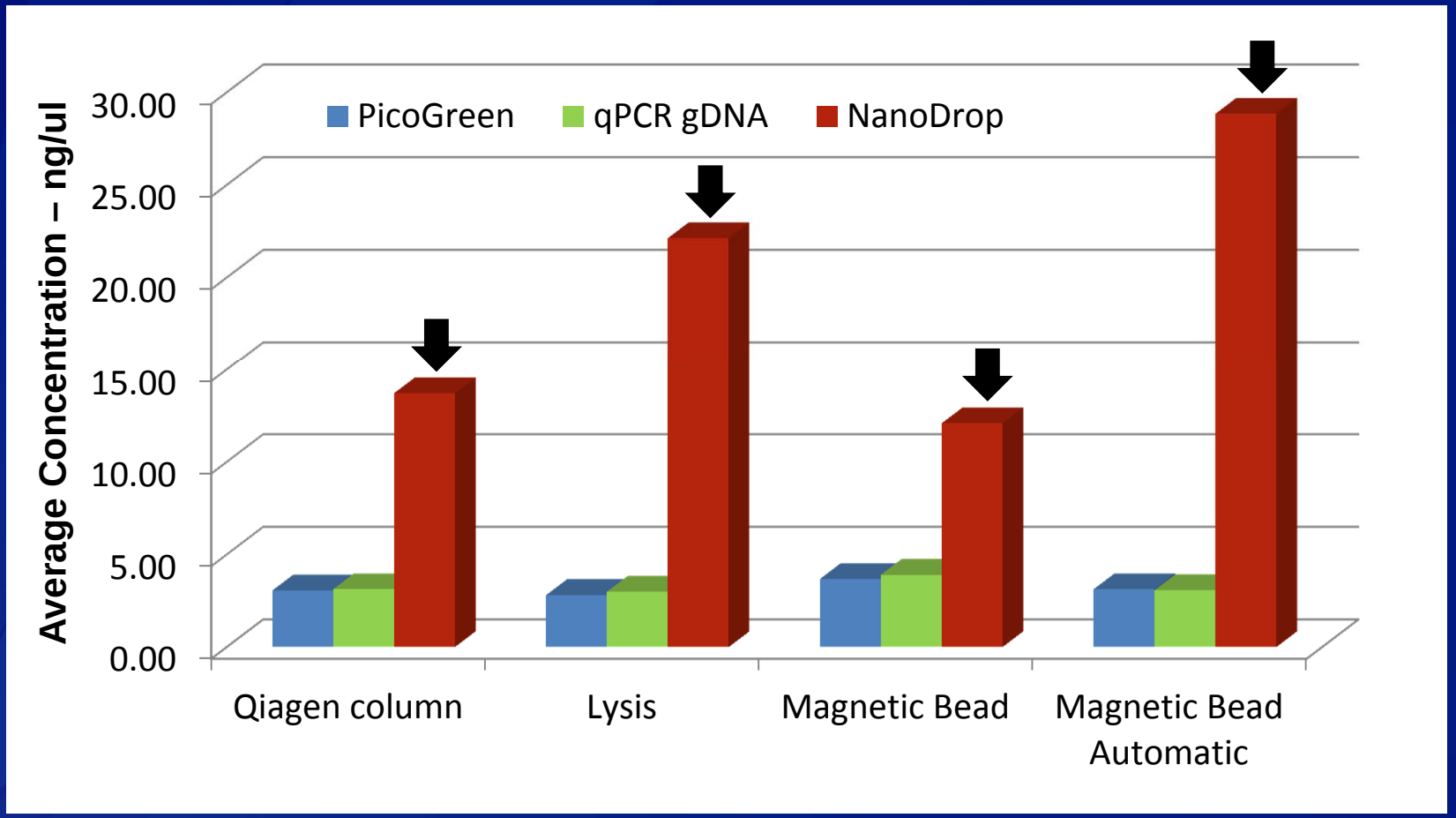
- Standard curve amplification – 8 points, each run in duplicate (pink curves)
- Unknown sample amplification – 4 samples, each run in duplicate (blue curves)

DBS DNA Extraction and Quantitation Study



*DNA was extracted from one 3mm punch

DBS DNA Quantitation Methods:
PicoGreen, NanoDrop and qPCR

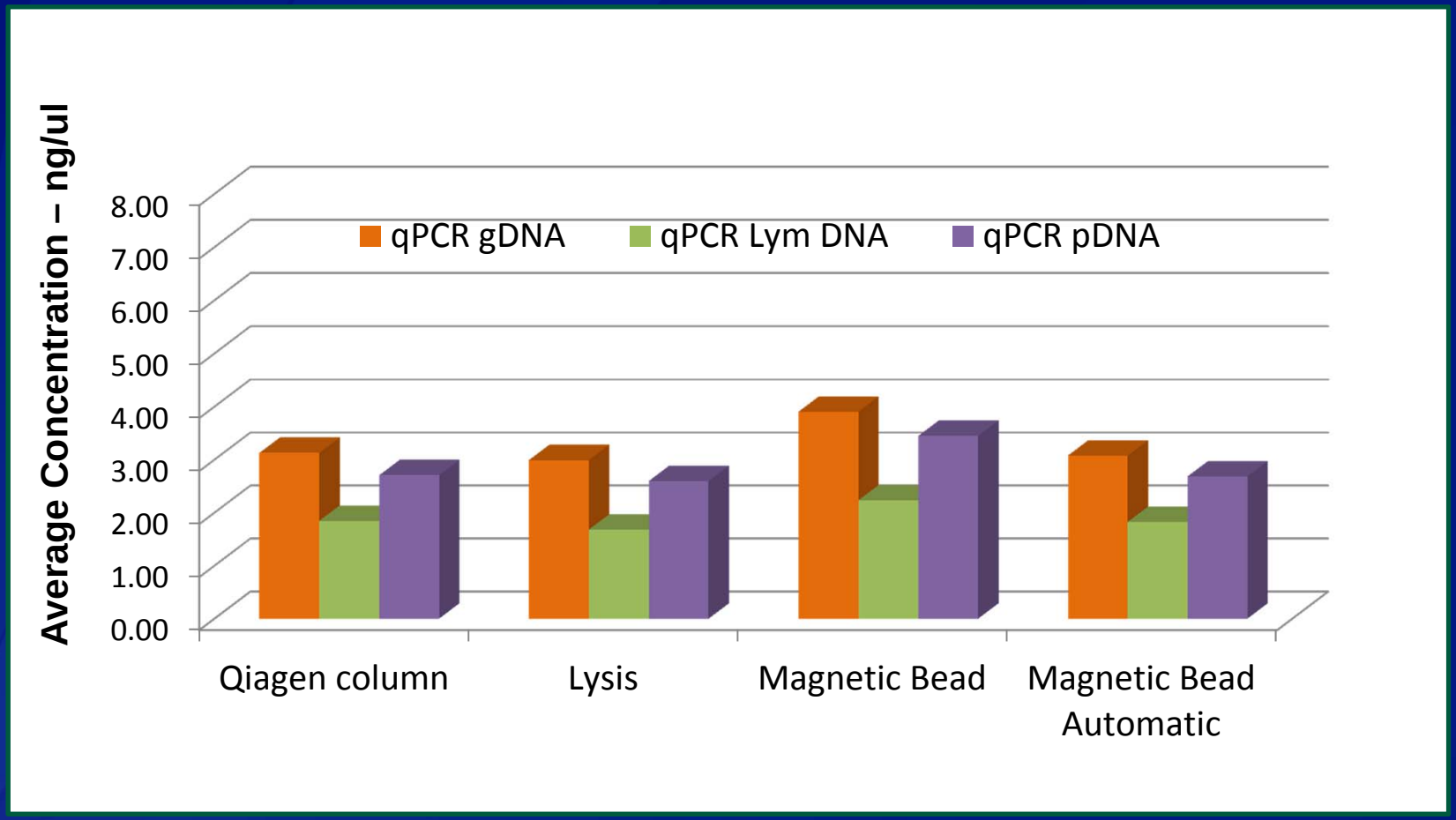


Average DNA Yield (ng) from each Extraction Method

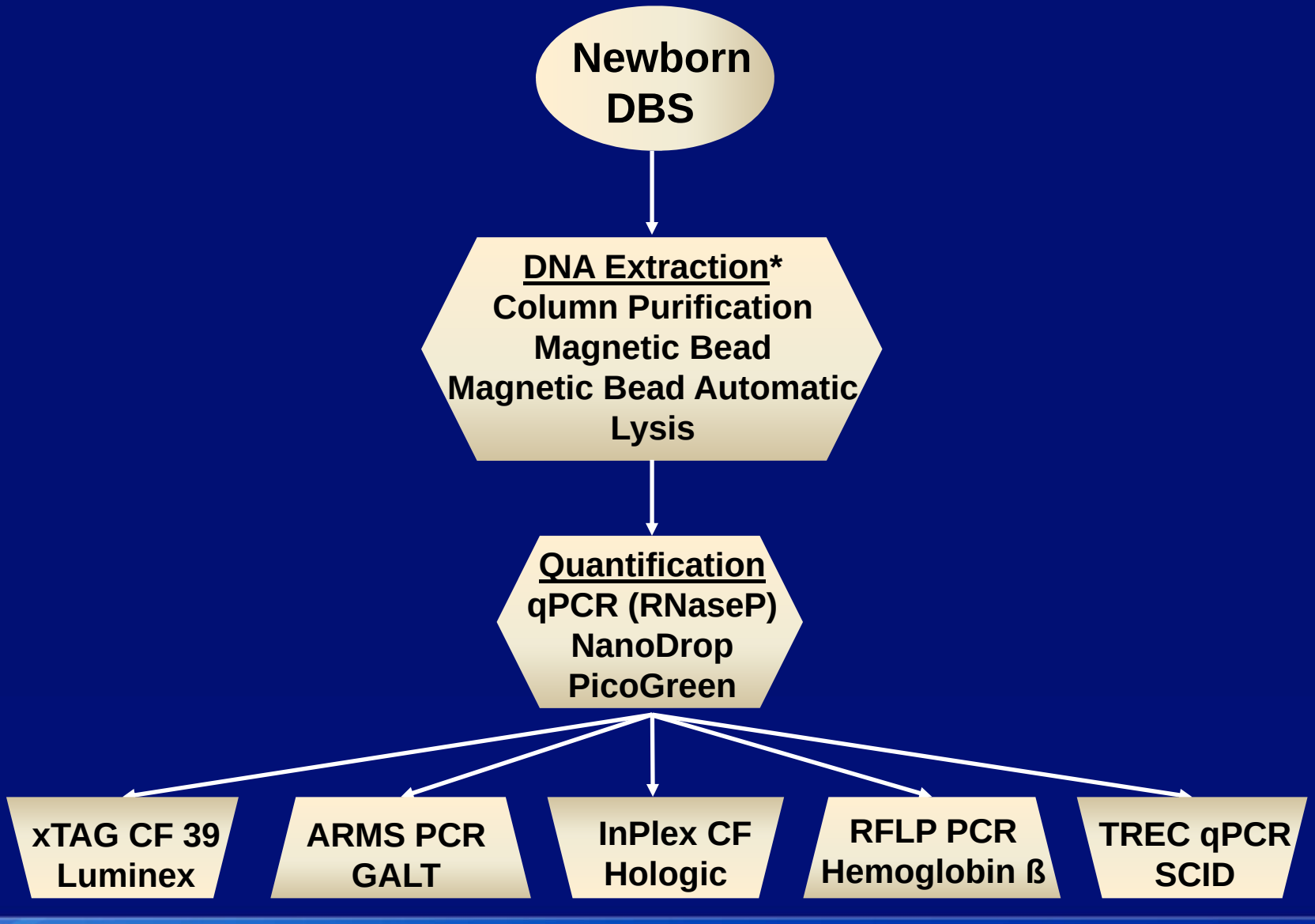
Extraction method	N*	PicoGreen (ng)	qPCR gDNA (ng)	NanoDrop (ng)
Column	20	183	188	[illegible]
Mag Bead	20	221	234	[illegible]
Mag Bead Auto	20	188	185	[illegible]
Lysis	20	169	180	[illegible]

*DNA was extracted from DBS that had been stored for 6 months at -20°C for 6 months

DBS DNA Quantitation qPCR: Using Different Standard Curve Materials



Evaluation of DBS DNA Extraction Methods



* DNA was extracted from one 3mm punch

DBS Extracted DNA Performance in Molecular NBS Assays

NBS Assay/Kit	# Mutations Detected	N	Col	Mag	MagA	Lys
xTAG CF 39 kit v2	39	20	0	16 (80)	6 (30)	0
Inplex CF	40	20	4 (20)	4 (20)	20 (100)	4 (20)
ARMS GALT PCR	1	20	0	0	20 (100)*	0
PCR-RFLP HbB	1	20	0	0	0	0
qPCR-TREC # TREC (Cq)	**	20	0 319 (31.8)	0 433 (31.2)	0 175 (32.7)**	0 244 (32.2)

*The ARMS GALT PCR assay failed on the 6 months DBS using the Magnetic bead automated extraction assay, however performed well of the 5 day old DBS extracted the same way.

**The TREC assay does not detect any mutations, rather the presence or absence of the T cell Receptor Excision Circle as well as an internal control.

DNA Extraction and Quantitation Study Conclusions

- DBS DNA quantitation using the NanoDrop overestimates quantity and is not suitable for DBS DNA
- qPCR performed with different standard curve sources does not perform the same and care should be taken when comparing yields
  - LYM DNA and pDNA source - 0.59 fold & 0.87 fold lower than gDNA respectively
- Column based and lysis DNA extractions performed consistently better in all NBS assays

DBS DNA Extraction and Evaluation Partners

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